The results described in this report were summarized in a manuscript which has been submitted for publication:

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Selective elimination/RNAi silencing of FMRFa-related peptides and their receptors decreases the locomotor activity in *Drosophila melanogaster*

(Gen. Comp. Endocrinology, submitted)

The above article as well as another one on the off-target effects will be published during the next two years. Therefore, we respectfully ask the OTKA Committee to take this fact into consideration when evaluating this closing report on OTKA Grant No. 75774.

Specific neurons in the central nervous system (CNS) secrete specific peptides which act as neurohormones steering vital processes in all Metazoans. Insects as well use a plethora of neuropeptides to control physiological, developmental and behavioral events (Nässel and Winther, 2010).We accomplished a genetic analysis in *Drosophila melanogaster* of the FMRF-related group of peptides (FARPs) comprising (DMS the dFMRFamide, dromyosuppressin) and drosulfakinin (DSK) peptides, and their specific receptors (FP, DMS-R1 and -R2, DSK-R1 and –R2).

In the original OTKA grant application, we planned to accomplish four different genetic approaches to study the FMRF-related (FARP) peptides and their specific receptors : (i) isolation of intragenic deletion mutants by P transposon remobilization; (ii) gene silencing by double-stranded inhibitory RNA (RNAi); (iii) induction of distinct mutations by homologous recombination in each of the 8 peptides coded for by the *FMRFa* gene; (iv) construction of UAS-cDNA transgenes for overexpressing the FARP peptides and receptors. We fulfilled the first two tasks, partially did the third one, and implemented a new (iv) project, i.e. construction of new Gal4 drivers with DNA fragments from the 5' regulatory sequences of the *FMRFa* gene. These changes were partly caused by the sudden and very sad death in 2011 of our paticipating colleague, Dr. Géza Ádám (Institute of Genetics, Biological Research Centre at Szeged of the Hungarian Academy of Sciences) who originally lead the molecular part of the work..

In the following sections, we give a detailed account on the experimental work and the results.

1. Isolation of intragenic deletion mutants for *FMRFa* and *DMS-R1*

Genomic insertions of the P element transposon can be remobilized by the specific transposase. The „jumping out" of the transposon causes a double-strand DNA break, and the repair process can result in short deletions. By P transposon remobilization, we isolated 8 independent intragenic deletions in the *dromyosuppressin-receptor 1* (*DMS-R1*) gene removing the first exon and partly the promoter region., and two deletions in the *FMRFa* gene, one deleting partly, the other entirely the peptide-coding region. Measuring the levels of the gene-specific mRNAs with RT-QPCR, we found deficiencies in both genes which apparenty eliminated the gene function. Despite of this, the homozygous mutant flies proved to be fully viable and fertile. In cooperation with Dr.Michal Žurovec (Inst. of Entomology, Czech Acad. Sci., České-Budejovice) we tested these mutants with respect to their basic metabolism (CO_2 production) and moving activity in a startle-induced behavioral test. In the latter test, repeated air-puffs pushed the flies to one end of a glass tube, after which a computerized camera followed their locomotor activity through 3 minutes, during which time the mean velocity of movement (MVM) values were calculated in short intervals. In the CO_2 production, there was no difference between mutant and wild type adults. However, the mutants showed significantly less moving activity than the wild type suggesting that the FMRFa peptide and the DMS-R1 receptor are needed for the normal activity.

2. RNAi silencing of FMRF-related peptides and receptors

We have built an RNAi-based genetic system in which the FMRFa-related genes and their specific receptors could be silenced in pairwise combinations. For this, we used the RNAi stocks available for every annotated *Drosophila* gene from the VDRC RNAi Stock Center (Vienna; http://stockcenter.vdrc.at) and the NIGFly collection (Kyoto; http://www.shigen.nig.ac.jp/fly/nigfly/index.jsp). In these stocks the RNAi transgene is driven by the Gal4-inducible UAS promoter. The test combinations contained an UAS-Dicer2 construct (on the X chromosome) to enhance the efficiency of the silencing effect, one or two UAS-RNAi constructs (2nd and/or 3rd chr., respectively) silencing different genes (a neuropeptide and/or its specific receptor or two related receptors) and a GAL4-

driver. We expected that the maximum silencing effect could be reached in combinations where the production of a neuropeptide and its specific receptor were simultaneously suppressed.

In the first experiment, the RNAi stocks were crossed to the *Act5C-Gal4* driver inducing a ubiquitous and continuous expression of the double-stranded RNA. As Table 1 shows, the silencing of certain genes resulted in complete lethality while others remained viable. The lethal effect was observed with the KK series of VDRC mutants where the silencing construct is inserted into a standard „landing platform" in the 2nd chromosome ensuring a uniform strong expression. The same constructs inserted in the 3rd chromosome at accidental places (GD series) had no lethal effect, probably due to lower expression

Because the main source of the neuropeptides are the neurons in the central nervous system (CNS), next we used the *elav-Gal4* pan-neuronal driver to induce the RNAi effect. As the *elav* promoter expresses the Gal4 exclusively in the neurons, the RNAi silencing is confined to the CNS. In comparison with the results gained with the *Act5-Gal4* driver, we hoped to decide which effect is dependent on the CNS and which comes from the periphery. We crossed the RNAi stocks listed in Table to the *elav-Gal4* driver. To our surprise, all these genetic combinations were viable and fertile.

Two logical explanations can be offered for this observation: (a) the lethality is induced by some ectopic (outside of CNS) expression of the RNAi effect induced by the *Act5C-Gal4* driver; (b) „off-target" RNAi effect, i.e. silencing non-specific genes which have sections in their DNA sequence similar to that of the target gene. In theory, neuropeptides can act outside of the CNS, and their receptors could be expressed on tissues on the periphery, but the data on the spatial expression of the receptors are rather limited. Therefore, we decided to examine the off-target effect in more detail.

Table 1: Phenotypic effects of RNAi silencing of the FMRFa-related peptides and their receptors

Silenced gene[1]	Construct I.D. number[2] (chromosomal location)	Genetic combination	F1 phenotype (25°C)[3]
FMRFa	KK 103981 (2)		**L**
FR	NIG 2114R-1 (3)		V
DMS	KK 108760 (2)		**L**
DMS-R1	KK101845 (2) GD 9154 (3)		**L**[4] V
DMS-R2	KK 109513 (2) GD 49953 (3)		n.d. V
DSK	KK 106592 (2)		V

DSK-R1	KK 102039 (2) GD 9154 (3)		V V
DSK-R2	KK 100760 (2) GD 7231 (3)		**L** V
FMRFa ; FR		103981;NIG 2114R-1	**L**
DMS ; DMS-R2		108760 ; 49953	**L**
DMS-R1 ; DMS-R2		101845 ; 49953	**L**
DMS-R2 ; DMS-R1		109513 ; 9370	**L**
DSK ; DSK-R2		106592 ; 7231	V
DSK-R1 ; DSK-R2		102039 ; 7231	V
DSK-R2 ; DSK-R1		100760 ; 9154	**L**

[1]Abbreviations:
n.d.=no data ; DMS=Dromyosuppressin peptide; DSK-R1,-R2=DMS-Receptor-1, -2;
DSK=Drosulfakinin peptide; DSK-R1, -R2=DSK-Receptor-1, -2;
FMRFa=FMRFa peptide; FR= FMRFa receptor; L=lethal; V=viable
[2]KK and GD label VDRC stocks (http://stockcenter.vdrc.at);
NIG labels NIGFLY stock (http://www.shigen.nig.ac.jp/fly/nigfly/indec.jsp)
[3]Crosses made at 18°C or 29°C gave results similar to those at 25°.
[4]Few short-lived escapers at 18°C with distorted wings and disorganized movement

We took the possible off-target genes referring to the „target" genes in Table 1, the RNAi silencing of which yielded lethality (FMRFa, DMS, DMS-R1, DSK-R2). For this we took the off-target genes as suggested in the VDRC stock-list. In addition, we made a BLAST search of the *Drosophila* genome for genes having at least 10 nucleotide long DNA sequence stretches identical with the target gene. As shown in Table 2, all the four target genes have several off-target counterparts, and crossed to the *Act5C-Gal4* driver the majority of the stocks gave lethality in the F1 generation. In fact, we found RNAi stocks for all the off-target genes which resulted in lethality in combination with the *Act5C-Gal4* driver.

Table 2: RNAi effects of off-target genes induced by *Act5C-Gal4*

Target gene	**VDRC Stock center**	**ID#**	**Off-target gene[1]**	**Off-target gene Transcripts[2]**	**RNAi effect induced by Act5C-Gal4 driver[3]**		
					VDRC RNAi stocks for off-target gene[4]	NIGFly RNAi stocks for off-target gene	Bloomington TRiP stocks for off-target gene
FMRF	Stock ID	*103981*	*Transport and golgi organization 1 (Tango1) CG11098*	1. Identities = 19/19 2. Identities = 13/13 3' UTR : none	**21594 (GD) 2**	**11098R-2** **11098R-3**	0
	RNAi construct ID	103629	*wishful thinking(wit) CG10776*	Identities = 22/24 (11/11) 3' UTR : none	**103808 (KK)** 865 (GD) **42244 (GD)** **42245 (GD)**	**10776R-1** **10776R-2**	**25949**
DMS	Stock ID	*108760*	*shaker cognate isoform G and I CG43128*	Identities = 11/11 3' UTR : none	13043 (GD) 46887 (GD) 46889 (GD) **47632(GD)**	0	**25805**

			discs large 1 (dlg1) CG1725	1. Identities = 17/19 2. Identities = 12/12 3' UTR: yes		**1725R-1** 1725R-2	25780 **31181** **31520** 31521 33620 **33629** **34854** 35286 35772
	RNAi construct ID	110955	*CG2201 isoform A and B*	Identities = 11/11 3' UTR yes	108958 (KK) 33502 (GD)	0	**35403**
			bride of sevenless protein gene (boss) CG8285	1. Identities = 16/18 2. Identities = 11/11 3' UTR : none	4365 (GD) 4366 (GD)	**8285R-1** **8285R-2**	29439)
DmsR-1	Stock ID	*101845*	*tenascin accessory, isoform E/H CG42338*	1. Identities = 15/17 2. Identities = 13/14 3. Identities = 12/12 3' UTR : none	**103298 (KK)** 105037 (KK) 8322 (GD) 32482 (GD) 39244 (GD) 39245 (GD) **40134 (GD)**	0	
	RNAi construct ID	109948	*musashi, isoform A/B (Msi) CG5099*	1. Identities = 11/11 2. Identities = 17/193' 3' UTR: yes (2.)	**11784 (GD)** **44895 (GD)**	0	0
			CG1105	Identities = 20/20 3' UTR : none	105981 (KK) **28305 (GD)** **28308 (GD)**	**1105R-1** **1105R-3**	31084 31224
DskR-2	Stock ID	*100760*	*ariadne-2(ari-2) CG5709*	Identities = 19/19 3' UTR : none	0	**5709R-1** 5709R-3	0
			CG3649	1. Identities = 21/22 2. Identities = 18/18 3' UTR : none	104762 (KK) 3517 (GD) **3518 (GD)**	**3649R-1** **3649R-2**	0
	RNAi construct ID	108482	*scratch (scrt) CG1130*	Identities = 23/25 3' UTR : none	**105201 (KK)** **41070 (GD)** **41071 (GD)**	**1130R-2** **1130R-3**	27025
			Elastin-like(Ela) CG7021	Identities = 22/24 3' UTR : yes	**107366 (KK)** **16474 (GD)**	7021R-2 7021R-3	0

Abbreviations: [1]CG: annotation number of the gene (http://flybase.org)
[2]Identities: section of perfect match (with the target gene) in the off-target nucleotide sequence (in the 3'UTR region: yes or not)
[3]Meaning of colours: **lethal;** viable; not tested)
[4]KK and GD: VDRC stocks with RNAi insertions ont he 2nd or 3rd chromosome, resp.

3. RNAi knock-down of the *FMRF* gene decreases the activity of the off-target companions

Table 3 shows the results of an experiment which directly tested the effect of silencing the *FMRF* gene on the mRNA levels derived from the *Tango 1* and *wit* genes. The RNAi sequence (664 nt) which codes for the dsRNA in the VDRC stock No. 103981 has two regions homologous with the *Tango 1* and *wit* genes, respectively. The *Tango 1*-homology is 19 nt long with a 13 nt sequence of perfect match. The 24 nt *wit* homology has an 11 nt sequence with 100% identity. There is no homology in the 3'UTR regions. When the No.103981 stock is crossed to the *Act5-Gal4* driver, the level of the *FMRF* mRNA drops to less than 1% of the control (Table 4, Templates 01 and 06, resp.). The output of *Tango 1* parallely drops to 47% albeit *wit* remains unchanged. When we silenced the *wit* gene (Template 03), *FMRF* and *Tango 1*outputs decreased to 19%.and 37%, respectively, so the effect is reciprocal.
In another experiment (Template 05, Table 4), the apoptosis-inducing *reape*r (*rpr*) gene was expressed by the *FMRF-Gal4* driver in the FMRF-producing neurons. This resulted in the death of these cells, and a serious decrease of the *FMRF* mRNA level. Again, this did not decrease (in fact, increased) the *wit* output. We have no data yet on the *Tango 1* level in these animals.

Table 3: Measuring off-target knock-down effect among the *FMRF*, *Tango 1* and *wit* genes by RT-QPCR

Template series	**log2 change**	**Expression %**
Template_01 *FMRF*	-6,9	0,837323
Template_01 *Tango 1*	-1,07	47,6319
Template_01 *wit*	-0,04	97,26549
Template_03 *FMRF*	-2,375	19,27764
Template_03 *Tango*	-1,425	37,24194
Template_03 *wit*	-2,305	20,23606
Template_05 *FMRF*	-2,63	16,15441
Template_05 *Tango 1*		n.d.
Template_05 *wit*	0,36	128,3426
Template_06 *FMRF*		100
Template_06 *Tango 1*		100
Template_06 *wit*		100

4. Creating single peptide-specific mutations in the FMRFa gene by homologous tecombination

We wanted to apply the S.I.R.T. method ("site-specific integrase mediated repeated targeting") described by Gao et al. (2008) for inducing mutations by homologous recombination in the individual peptide-coding sequences of the *FMRFa* gene. In short, at first an *attP* attachment site for the phage phiC31 integrase is inserted in the vicinity of the gene of interest by homologous recombination. After that any modification of the gene can

be introduced by phiC31 integrase-mediated insertion of plasmids carrying an *attB* attachment site and the desired mutation. The integration results in a tandem duplication of the target locus, which has to be reduced to a single copy carrying the mutation, during the repair process induced with a DNA double-strand break. Although this work was slowed down by the other experiments (see later and above), and the very sad death of our late colleague and molecular biology expert Dr. Géza Ádám, we finished introducing the phiC31-specific *attP* landing sequence within the first intron of the *FMRFa* gene. However, we had to stop with the work at this point.

5. Selective elimination/RNAi silencing of FMRFa-related peptides and their receptors decreases the locomotor activity in *Drosophila melanogaster*

Previous studies have already suggested that the FaRP peptides and receptors have myotropic effects (Nichols et al., 2002; Kahsai et al., 2010). The experiments described here address the effects exerted by the FaRP peptides on the locomotor activity of *Drosophila* adults. For this purpose, we used two genetic approaches: (i) RNAi silencing of FMRF-related peptides and their receptors (see above) and (ii) construction of new FMRF-Gal4 drivers which represent specific parts of the FMRFa spatial expression in the CNS, and their application to remove the corresponding neural cells by *reaper* (*rpr*)-induced apoptosis.

5.1.Construction of FMRFa expression pattern-specific Gal4 drivers

Benveniste and Taghert (1999) described 5' upstream DNA sequences regulating the spatial expression in the CNS of the *FMRFa* gene. When they cloned these DNA fragments into a vector upstream of the β-galactosidase gene, they found the staining pattern reproducing specific parts of the FMRFa spatial expression. Based on this information, we amplified three such DNA sequences by PCR, cloned them into the *pBPGUw* vector upstream to the Gal4 coding sequence, and made transgenic *Drosophila* strains (*RS8-Gal4, RS11-Gal4, RS17-Gal4*) carrying these constructs on the 3rd chromosome in the *attP* „landing platform" providing a strong and uniform expression. By making use of the *UAS-GFP* reporter gene, we tested the Gal4 expression in these stocks. As it was shown by the GFP fluorescence and GFP-specific immuno-staining, the expression pattern in the larval CNS of RS8, RS11 and RS17 reproduced the patterns described by Benveniste and Taghert (1999) for the same regulatory sequences

(Fig. 1). However, additional GFP-positive neurons were also detected in all the three cases, especially in RS8. This might be explained by the enhanced visibility of the GFP expression provided by the immuno-staining with GFP-specific antibody. Another difference between our constructs and those of Benveniste and Taghert (1999) was in the relative orientation of the cloned sequences. In all three constructs of ours the orientation of the regulatory DNA segment relative to the Gal4 coding part was the same as in the genomic sequence. In the case of Benveniste and Taghert, however, the regulatory sequences corresponding to RS8 and RS11 were cloned in the reverse orientation. Benveniste and Taghert attributed the observed expression patterns to the effect of enhancers, the activity of which is not influenced by their relative position and polarity. However, the possible effect of other „non-enhancer" sequences in the cloned DNA segments might be sensitive to their polarity relative to the coding region, and this could also be responsible for the ectopic expression. Unraveling this problem needs further studies.

5.2. RNAi silencing of FaRPs and FaRP receptors in the CNS can decrease locomotor activity

Flies having the RNAi constructs for a FaRPs and their specific receptors were crossed to the *elav-Gal4* driver strain. The offspring carrying the RNAi transgenes and the *elav* driver showed normal viability and fertility, and was subjected to the ReSH (Repetitive Startle-induced Hyperactivity) test as described by Lebetsky et al. (2009). Briefly, the flies were transferred into glass tubes connected to a pressurized air container (5 bars). Then two air puffs were delivered in a 1 sec. interval which pushed the flies to the rear end of the tube closed with a fine mesh. The video-recording began 1 min. before the first air puff and lasted for 4 min. afterwards. To avoid the differences generated by the cyrcadian rhytm, the tests were made in late morning or early afternoon. Three cycles were made with each group of flies. Four experiments (each one repeated in 3 cycles) were performed with different flies of each genotype, i.e. 80 male or female flies for every genotype were tested altogether. Based on the video records, the average velocity (mm/sec) of the flies was calculated by the *Drosana* custom computer program (developed by Stefan Kakaš) in 0.1-0.2 sec intervals, and used to construct curves representing the changing mean velocity of movement (MVM) in time.

The results are shown in Fig. 1 for the female flies. (Similar effects were observed with the males, data not shown.) In general, suppressing the FARPs and/or their receptors

significantly decreased the mean velocity of movement (MVM) of the flies in all genetic combinations tested. The MVM decrease was manifested at both the resting activity before the air puffs, and the puff-induced activity. The largest difference between the RNAi-reduced MVM and the control values was found when both DMS-receptors (DMS-R1 and DMS-R2) were silenced in parallel. As for the absolute values, three of the tested combinations (silencing transgenes on the 2nd; 3rd chromosomes: *RNAi-DMS/elav;RNAi-DMS-R2/+*, *RNAi-DMS-R1/elav;RNAi-DMS-R2/+*, *RNAi-DSK/elav;RNAi-DSK-R2/+*) showed the largest MVM decrease. In the two other cases (*RNAi-FMRFa; RNAi-FR* and *RNAi-DMS-R2; RNAi-DMS-R1*) the MVM decrease was less. For the *RNAi-DSK-R2; RNAi-DSK-R1* silencing combination, the difference from the controls was not so remarkable.

As mentioned above, the tested transgenes on the 2nd chromosome (KK series of the VDRC Stock Center) are inserted in the *attP2* platform and have an uniformly strong expression, while the transgenes on the 3rd chromosome (GD series) have a varying, probably weaker expression due to the varying position effects. Although both the *RNAi-DMS-R1/elav-Gal4;RNAi-DMS-R2/+* and *RNAi-DMS-R2/elav-Gal4;RNAi-DMS-R1/+* combinations silenced the *DMS-R1* and *DMS-R2* genes together (Fig. 1, C and D), the MVM effect was stronger when the *RNAi-DMS-R1* transgene was inserted in the *attP2* platform on the 2nd chromosome and the *RNAi-DMS-R2* on the 3rd (Fig. 1, C and D). The difference in the chromosomal position effects may be responsible for the variation between the MVM curves. These results show that, at least in the combinations tested, the FaRPs and their receptors can modify the locomotion activity and stress response of the fruitfly adults.

5.3. Selective elimination of FMRFa peptiderg neurons in the CNS and its effect on the locomotor activity.

To induce the targeted elimination of FMRFa-positive neurons, males of the *RS8, RS11, RS17* and *FMRF-Gal4* driver stocks were crossed to females of the *UAS-rpr* (X chromosome) transgenic strain. The elevated expression of the wild-type *rpr* gene induced apoptotic death of the neural cells (cf. Table 3, Template 5). The offspring carrying the *RS11, RS17* or *FMRF-Gal4* drivers together with *UAS-rpr* developed normally, and resulted normal adults. However, a good part of the *UAS-rpr/Y;RS8/+* males and *UAS-*

rpr/+;RS8/+ females died as pharate adults. About 20% of the surviving flies had distorted wings, moved uncoordinately, and died within 2-3 days after eclosion.

The flies transheterozygous for the *Gal4* drivers and *UAS-rpr* were subjected to the ReSH test. The results are shown in Fig.3 for the females. Of the *UAS-rpr/+; RS8-Gal4/+* females, only flies with normal wings were selected for the test. As Fig.3A shows, these flies showed a low pre-puff activity which was only slightly elevated by the air puffs. Interestingly, the usual MVM peak instant with the puffs was missing in this case. Examining the video-recordings showed that the flies mostly flocked together at the rear end of the tube, and did not move much upon and following the air puffs. On the contrary, the activity of the *UAS-rpr/Y;RS8-Gal4/+;* males was unexpectedly close to the control (not shown). This would mean that the *RS8-Gal4*-induced neuronal ablation interfered with the regulation of the startle-induced locomotor response in some gender-specific way.

We further examined the locomotor activity of the „sluggish" *UAS-rpr/+; RS8-Gal4/+* females in the negative geotaxis test. Briefly, the flies were transferred into glass tubes of vertical position, and were shaken off by knocking the rack twice to the table manually. Leaving the tube in vertical position, photographs were taken after 10, 20 and 30 seconds, and the flies were counted according to their distribution by height along the tube.

The results are shown in Fig. 4. Interestingly, the flies were just as active as the controls. This may mean that the startle-induced locomotor activity and the negative geotaxis are differently regulated in the *UAS-rpr/+; RS8-Gal4/+* female flies.

The other female combination which showed a remarkable decrease in the puff-induced MVM was *UAS-rpr/+;RS17/+* (Fig. 3). As for the remaining two combinations, *UAS-rpr/+;RS11/+* and *UAS-rpr/+;FMRF-Gal4/+* coincided with the control activities (Fig. 3, B and D, resp.).

In the male combinations, *UAS-rpr/Y;RS8/+* and *UAS-rpr/Y;RS11/+* were not significantly different from the controls. The *UAS-rpr/Y;RS17/+* combination only moderately decreased the MVM, while the behavior of *UAS-rpr/Y;FMRF-Gal4/+* flies was indistinguishable from the controls.

The above experiments resulted in several unexpected observations. First of all, the completely normal activity and behavior of the flies carrying the *UAS-rpr* and *FMRF-Gal4* transgenes was a surprise. In these flies all the FMRF-producing neurons are most likely missing, consequently the level of the FMRFa peptides is very low (see Table 3, Template 5). (It has to be noted, however, that in this case the activity of the FR receptor is still normal.) On the other hand, when the *RS8, RS11* and *RS17* drivers were used to drive the

expression of *UAS-rpr*, only specific parts of the FMRF-expressing neuronal pattern were ablated, and yet it influenced the flies' locomotor activity (see 5.3. as well as Fig. 3). Also, when the genes coding for the FMRFa peptides and the specific FR receptor were silenced together, the MVM of the flies became much lower than that of the control (see 5.2. and Fig. 2). In the light of these findings, the ineffectiveness of the *UAS-rpr;FMRF-Gal4* combination, where the FMRFa-producing neurons have been killed, is enigmatic. One can speculate that the neurons activated in the *FMRF-Gal4* expression pattern are in some kind of balance, so when the whole pattern is missing, the lacking parts could neutralize each other's effects. This notion may be supported by the observation that in the *RS8, RS11* and *RS17* drivers the Gal4 is expressed in extra neurons which are outside of the previously described (Benveniste and Taghert, 1999) FMRF pattern, and might interfere with this hypothetical balance leading to the low MVM phenotype. In this respect, the *RS8* driver activates most of the extra neurons (Fig.1, D). Furthermore, in the *UAS-rpr;RS8* combination the high incidence of pharate adult lethality and the distorted wing phenotype suggest that the *RS8* driver can express the Gal4 in other tissues as well outside of the CNS, e.g. in the wing imaginal disc epithelium. All these may mean that if a partial sequence is taken out of the original context of a regulatory region, ectopic new activities may arise.

Closing remark: The above results were summarized in a manuscript which has been submitted for publication:

> Brigitta Kiss, Tamás Szlanka, Beáta Ramasz, Zoltán Hegedűs, Michal Žurovec, Michal Sery, Štefan Kakas, Tamás Lukacsovich, Adrien Fónagy and István Kiss:
>
> **Selective elimination/RNAi silencing of FMRFa-related peptides and their receptors decreases the locomotor activity in *Drosophila melanogaster***
> **(Gen. Comp. Endocrinology, submitted)**

The above article as well as another one on the off-target effects will be published during the next two years. Therefore, we respectfully ask the OTKA Committee to take this fact into consideration when evaluating this closing report on OTKA Grant No. 75774.

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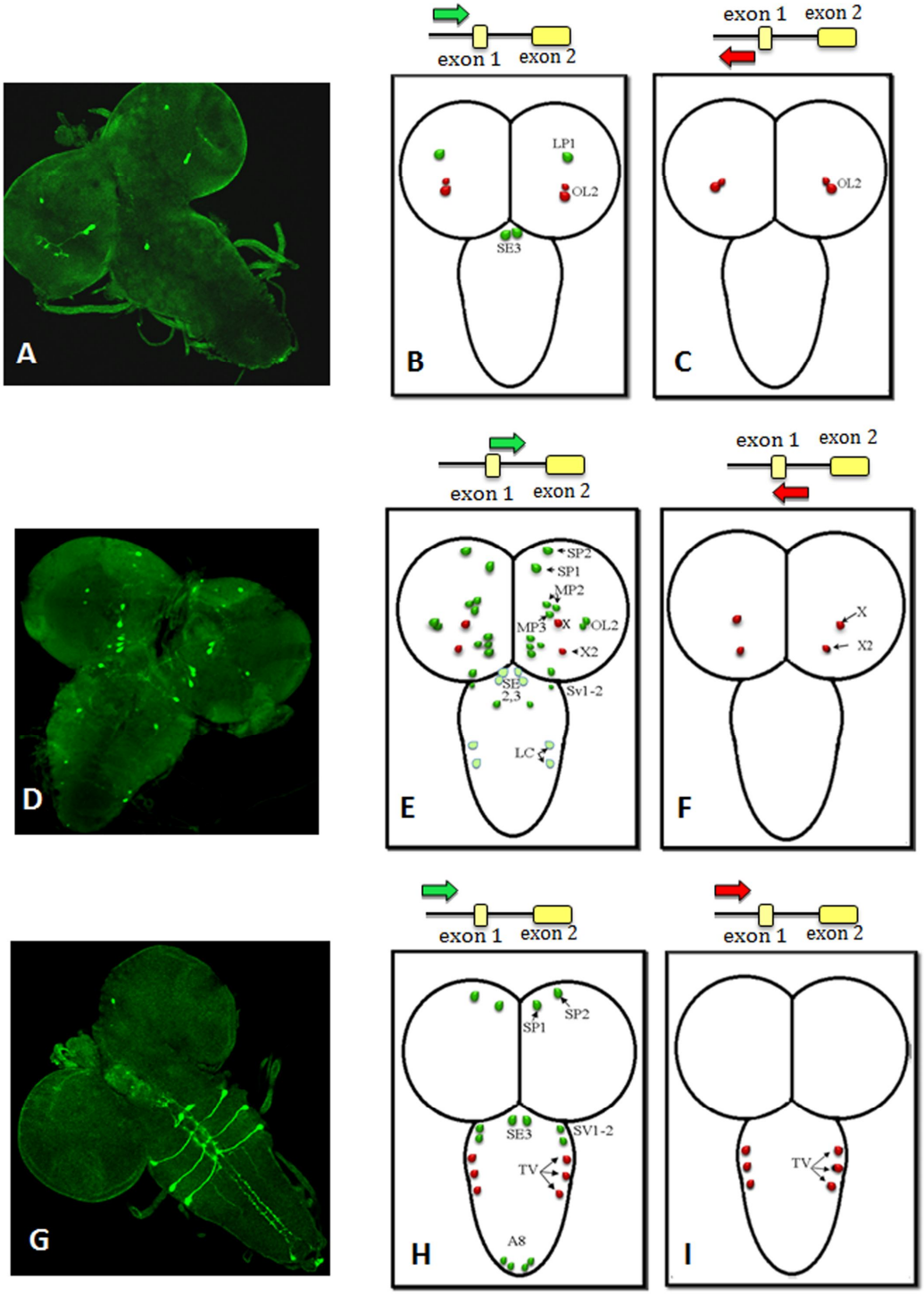

Figure1: Transcription activating patterns of different regulatory sequences (A-C: RS11, D-F: RS8 and G-I: RS17) of the *FMRFa* gene in the larval CNS. Confocal micrographs (A, D and G) and their schematic representations (B, E and H) of immuno-stained UAS-GFP expressing cells in the brain and the ventral ganglia of third instar larvae. Schematic representations of beta-galactosidase expressions (C, F and I) in larval CNS, controlled by RS11, RS8 and RS17 (after Benveniste and Taghert, 1999). Positions of the

regulatory sequences within the *FMRFa* gene 5' region and their orientations relative to the reporter genes in the two different types of testing constructs are depicted above each schematic panel. In the schematics **red** and **green** points represent FMRFa-positive neurons described by Benveniste and Taghert (1999) and/or observed in this study, respectively.

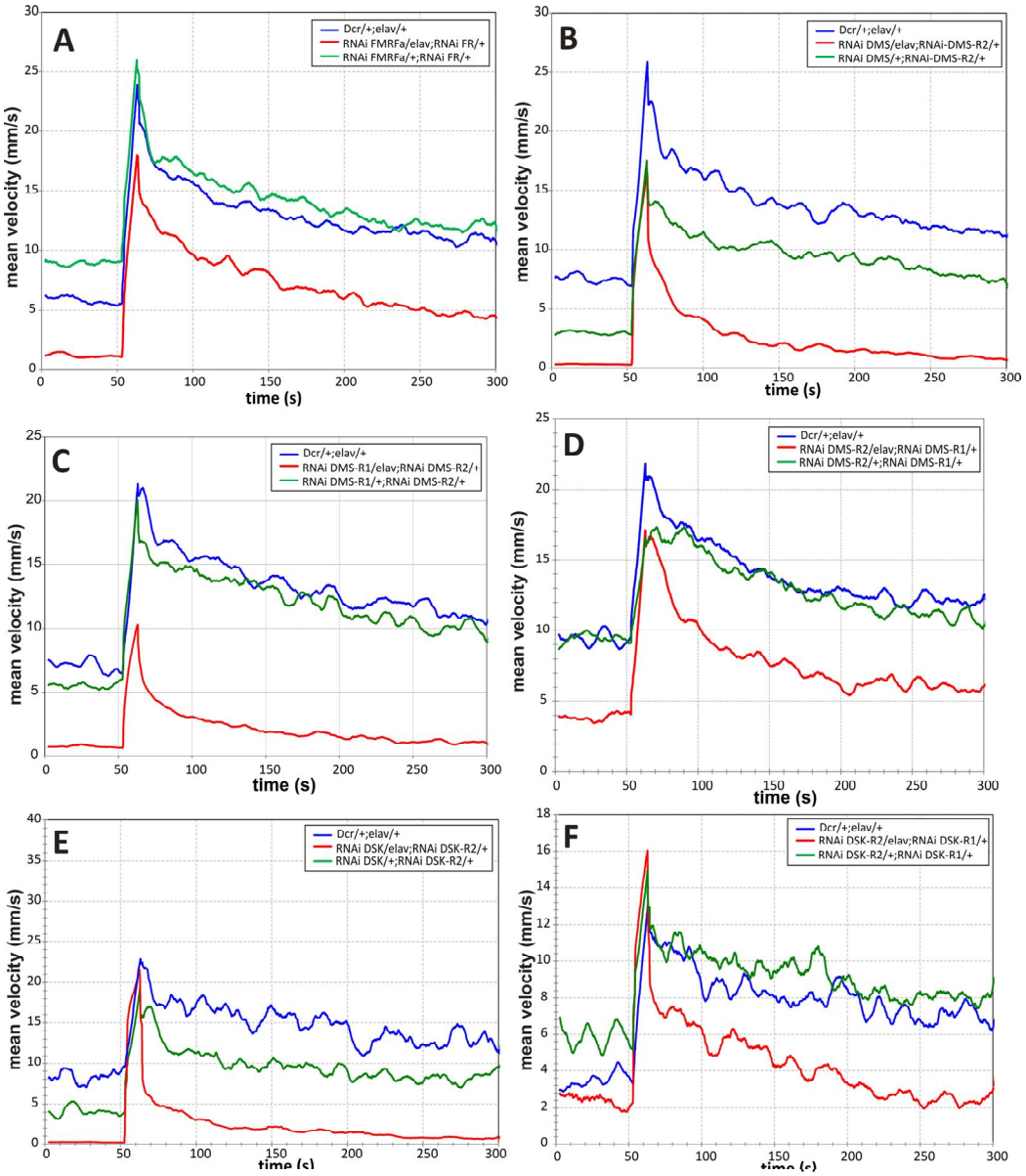

Figure 2: RNAi knock-down of FaRP and FaRP-receptor genes effectively changes the startle (air puff)-induced locomotor activity of *Drosophila* females. The curves represent the changes in time of the mean velocity of movement (MVM) before and after the air-puffs. **Blue** and **green**: control curves; **red**: RNAi knock-down curve.Fly genotypes are indicated in the upper right corner of each panel. (Abbreviations: Dcr=Dicer; i=UAS-RNAi, Gal4-inducible transgene producing double-stranded RNA; elav=*elav-Gal4* driver)

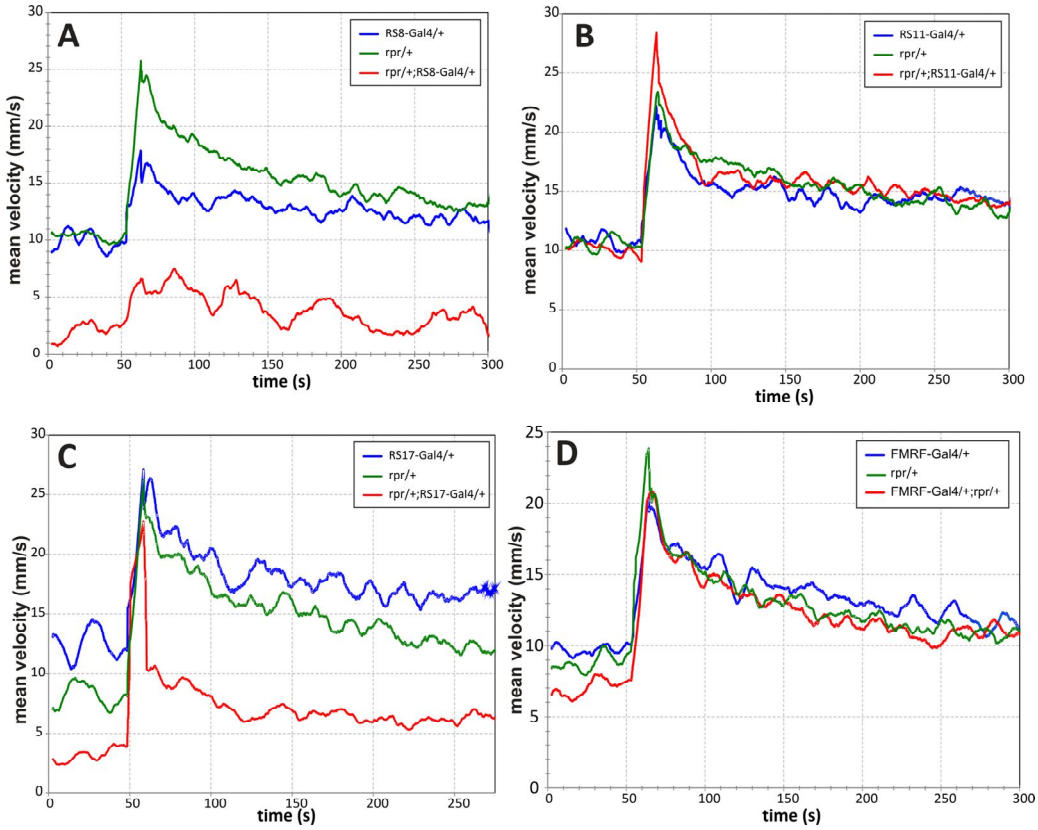

Figure 3: Ablating subsets of FMRFa-producing neural cells in the CNS may influence the startle (air puff)-induced locomotor activity of *Drosophila* females. The ablating effect is targeted by RS-Gal4 drivers which induce the apoptotic *UAS-rpr* transgene. The curves represent the changes in time of the mean velocity of movement (MVM) before and after the air-puffs. **Blue** and **green**: control curves; **red**: ablation effect curve. Fly genotypes are indicated in the upper right corner of each panel. (Abbreviations: RS8, RS11, RS17=regulatory sequences from the 5' region of the *FMRFa* gene; rpr=*UAS-reaper* transgene.)

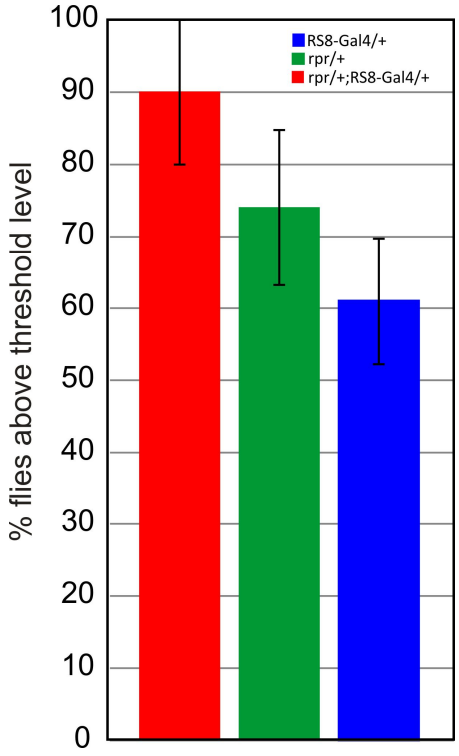

Figure 4: *RS8-Gal4*-induced ablation of specific FMRFa-producing neurons does not affect the negative geotaxis of female flies. The bars represent the average number of flies climbed up above the 95 mm threshold level. **Blue** and **green**: controls; **red**: ablation effect. Fly genotypes are indicated in the upper right corner. (Abbreviations: RS8=regulatory sequence from the 5' region of the *FMRFa* gene; rpr=*UAS-reaper* transgene.)